The Endothelium in Clinical Practice

Source and Target of Novel Therapies

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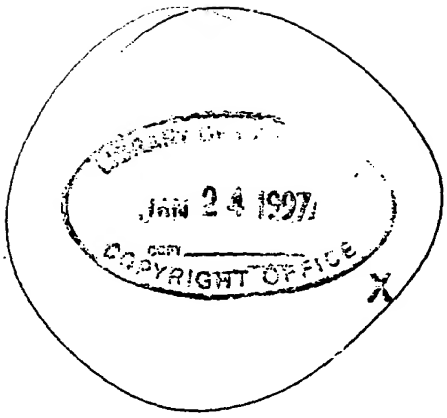

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The Receptor for Advanced Glycation Endproducts: Implications for the Development of Diabetic Vascular Disease

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I. INTRODUCTION

Proteins or lipids exposed to aldose sugars undergo nonenzymatic glycation and oxidation (1–7). The ultimate products of these interactions are called the advanced glycation endproducts (AGEs). Earlier, however, reversible adducts known as Schiff bases/Amadori products form in the course of exposure to aldoses. These products, distinct from AGEs, are reversibly formed and can dissociate to the native proteins on restoration of normoglycemia (4). Clinically, the reversible products have been used to assess the adequacy of glycemic control. The best known of these moieties used in this setting is hemoglobin A_{1c} (8). In contrast, AGEs are irreversibly formed. Although a heterogeneous group of compounds, they nevertheless share in common certain characteristics such as yellow-brown color, the tendency to form cross links, the ability to generate reactive oxygen intermediates, fluorescence, and the ability to recognize specific receptors on cellular surfaces (9–13). AGEs accumulate in the plasma and vessel wall during normal aging, but they form to an accelerated degree in patients with diabetes.

A number of groups have developed specific reagents (both polyclonal and monoclonal antibodies) to detect the presence of AGEs in vivo (14–17). For example, we have identified increased immunoreactivity for AGEs in the vasculature of diabetic patients versus that in age-matched,

nondiabetic control individuals (18). Similarly, others have found increased immunoreactivity for AGEs in the vessel wall and skin of diabetic patients (19–22). The results of these studies are consistent with the hypothesis that formation of AGEs correlates with the development of diabetic complications. As well, previous studies have suggested a possible link between the accumulation of AGEs in the vasculature and the development of vascular disease which occurs to an accelerated extent in diabetic individuals. For example, Nakamura and colleagues demonstrated increased immunoreactivity for AGEs in coronary atheromas of patients with diabetes (22).

It should be noted that in addition to normal aging (1,2,4) and diabetes (1–8), the development of proteins/lipids modified by irreversible nonenzymatic glycation may occur under other conditions which favor their generation. For example, AGEs may form on long-lived proteins, particularly in the setting of renal failure. In this context, under physiological conditions, the plasma protein β_2-microglobulin is readily cleared from the circulation. However, in the presence of its markedly delayed clearance characteristic of renal failure, β_2-microglobulin may undergo AGE formation (23), presumably as a consequence of its prolonged half-life in the setting of renal failure. In marked contrast to the native form, AGE–β_2-microglobulin is postulated to be important in the pathogenesis of the inflammatory disorder dialysis-related amyloidosis (DRA) that is associated with chronic renal failure and dialysis (24).

Another condition in which amyloid deposition may predispose to the development of AGE formation is in Alzheimer's disease (25). In this disorder, AGE formation may form on the components of intracellular neurofibrillary tangles (26–28) as well as in extracellular amyloid–β-peptide accumulations (29). The presence of these AGE-modified moieties has been postulated to be an important component of enhanced oxidant stress, which is likely involved, at least in part, in the pathogenesis of this disorder (26,28).

Taken together, these data suggest that AGEs may form under conditions of hyperglycemia, normal aging, delayed protein turnover (such as in renal failure), or extensive protein accumulation (such as in amyloidoses). In this chapter, we detail some of the biological properties of these structures with respect to their likely contributory role in the development of vascular/cellular pathology.

II. IDENTIFICATION OF CELLULAR RECEPTORS FOR AGEs

Earlier studies identified the ability of AGE-modified ligands to bind to cellular surfaces (4,30) in a specific and saturable manner. Although a number of cellular binding sites for AGEs have been identified (11,31,32), the

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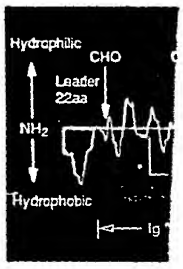

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best characterized of these to date is the receptor for advanced glycation endproducts (RAGE) (11). By sequential chromatography on hydroxylapatite, fast-pressure liquid chromatography (FPLC) Mono S, and FPLC gel filtration media, RAGE was purified to homogeneity from detergent extracts of bovine lung. At the same time, another AGE binding polypeptide, lactoferrin-like polypeptide (LF-L) was also described (11). This chapter is confined to consideration of RAGE.

Molecular cloning studies (13) have revealed that RAGE is a newly identified member of the immunoglobulin superfamily of cell surface molecules. The predicted hydropathy plot of the bovine cDNA for RAGE (Fig. 1) suggests that RAGE contains a 332–amino acid extracellular domain with one "V"-type immunoglobulin domain followed by two "C"-type domains. This is followed by a hydrophobic transmembrane spanning domain and a highly charged cytosolic tail. Preceding the above domains is a putative signal sequence of 22 amino acids. Of note, the bovine, murine, rat, and human counterparts of RAGE are remarkably similar. Critical to the hypothesis that RAGE was an AGE binding protein was the demonstration of binding of ^{125}I-AGE albumin to 293 cells transfected with the cDNA for RAGE but not to cells transfected with irrelevant cDNA (13). Binding to RAGE-transfected 293 cells was similar to that of radiolabeled AGE albumin to immobilized RAGE, endothelial cells, or mononuclear phagocytes (11,13,16) with $K_d \approx 50$–100 nM and inhibitable in the presence of anti-RAGE IgG (11,13,16).

Given the fact that RAGE was a member of the immunoglobulin superfamily, we postulated that under physiological conditions, RAGE might

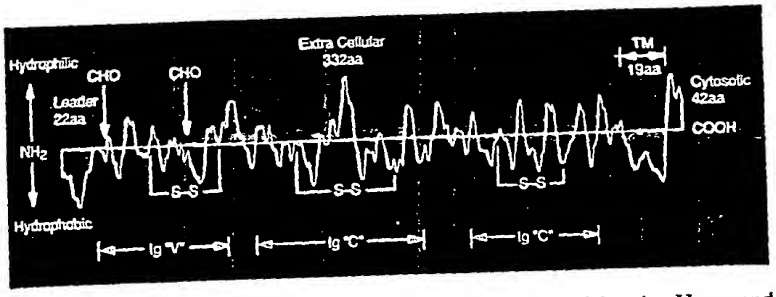

Figure 1 Hydropathy plot of bovine RAGE. As generated by the Hopp and Woods program of intelligenetics, this hydrophilicity plot of RAGE was generated. NH_2, amino terminus; COOH, carboxyl-terminus; CHO, N-linked glycosylation sites; TM, transmembrane domain; S–S, disulfide-linked cysteine residues; IgV and IgC, immunoglobulin-like variable and constant domains, respectively.

function as a cell-cell adhesion molecule or receptor for cytokine(s) or growth factor(s). In this context, we have demonstrated that under the specific condition of perinatal development, RAGE is highly expressed in the developing central nervous system of the rat. Its specific interaction with the polypeptide amphoterin appears to mediate neurite outgrowth in in vitro assays designed to study this phenomenon (33). Another recently identified putative ligand of RAGE, a molecule with $M_r \approx 12$ kD, is a newly identified member of a family of proinflammatory cytokines (33). Detailed analysis of the consequences of its interaction with RAGE are in progress.

In addition to the presence of RAGE on endothelial cells and mononuclear phagocytes, a survey of normal tissue has identified its presence on other cell types such as mesangial cells and certain neurons (34). In addition, RAGE is also present in the vascular smooth muscle (34). As stated above, RAGE is also present to a significant degree in the developing nervous system in embryonic and postnatal (through P17) cortical neurons (33). These data lend further support to the hypothesis that AGEs may in fact be pathological ligands of this receptor, which under homeostatic conditions, act to mediate such functions as normal development and, potentially, other not yet identified phenomena.

In this chapter, we concentrate on the implications of AGE-RAGE interaction on mononuclear phagocytes and endothelial cells.

III. INTERACTION OF AGEs WITH MONONUCLEAR PHAGOCYTE RAGE: INITIATION OF MONONUCLEAR PHAGOCYTE MIGRATION AND ACTIVATION

Previous studies have identified the presence of RAGE on mononuclear phagocytes by immunohistochemistry (16) and by in situ hybridization (S.D. Yan and A.M. Schmidt, unpublished observation). Radioligand binding studies with ^{125}I-AGE albumin demonstrated specific saturable binding, with $K_D \approx 80$ nM, comparable to that observed on immobilized RAGE (11). Consistent with the concept that RAGE was important in mediating the interaction of AGEs with mononuclear phagocytes (MPs), binding was largely blocked in the presence of anti-RAGE IgG.

We hypothesized that soluble AGEs, such as those found circulating on modified plasma proteins or on the surface of diabetic red blood cells (35), for example, might initiate monocyte migration. In this context, when soluble AGEs (either AGE albumin or AGEs immunoisolated from diabetic plasma using affinity-purified anti-AGE IgG) were placed in the lower compartment of microchemotaxis chambers, monocyte migration was promoted in a dose-dependent manner (16). In contrast, native albumin was without effect. Furthermore, that this was true chemotaxis was demonstrated by

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further studies with checkerboard analysis. Similar results were observed with AGE–β_2 microglobulin with respect to true monocyte chemotaxis (24). Migration of monocytes in response to soluble AGE albumin was inhibited in the presence of anti-RAGE IgG (or F[ab']$_2$ fragments) or in the presence of soluble RAGE (sRAGE, the extracellular two thirds of the molecule [16]). In contrast, blockade of RAGE was without effect on monocyte migration initiated by the chemotactic peptide F-met-leu-phe (FMLP).

In addition to soluble AGEs, AGEs may also form on the long-lived proteins of the subendothelium, an area postulated to have an important role in the development of vascular lesions, such as atherosclerosis (36). In order for the AGEs formed/deposited in this area to be of pathological significance, they would likely need to attenuate monocyte migration. To study this hypothesis, we immobilized either AGE albumin or native albumin on the upper membranes of microchemotaxis chambers and tested monocyte migration in response to the chemotactic peptide FMLP placed in the lower compartment. When the upper surface was coated with native albumin, monocyte migration in response to increasing doses of FMLP was increased, as expected. However, when AGE albumin was immobilized on the upper surface, monocyte migration in response to FMLP in the lower compartment was attenuated in a dose-dependent manner (16). The central role of monocyte RAGE in mediating these interactions was demonstrated in studies employing RAGE blockade. When monocytes were preincubated with anti-RAGE F(ab')$_2$, monocyte migration in response to FMLP was restored despite the presence of immobilized AGE on the upper surface. In contrast, nonimmune fragments were without effect. Similarly, when the AGE-coated surface was pretreated with sRAGE, monocyte migration in response to FMLP in the lower compartment was restored (16).

To further test the hypothesis that when migrating MPs encounter immobilized RAGE, their migration is slowed, we performed phagokinetic track assays directly to visualize monocyte migration on either AGE albumin or native albumin–coated surfaces. When MPs were placed on coverslips coated with native albumin that had been overlaid by colloidal gold, their migration was evident with long paths of migration demonstrated (Fig. 2, left panel). In marked contrast, when MPs were placed on coverslips coated with AGE albumin, their migration was slowed, as evidenced by short paths of migration (Fig. 2, right panel).

Analogous experiments were performed in vivo to further demonstrate this concept. Polytetrafluoroethylene (PTFE) tubes were impregnated with either native rat serum albumin or AGE rat serum albumin and then implanted subcutaneously onto the backs of rats. When removed after 4 days, the tissue of rats treated with PTFE tubes to which had been absorbed

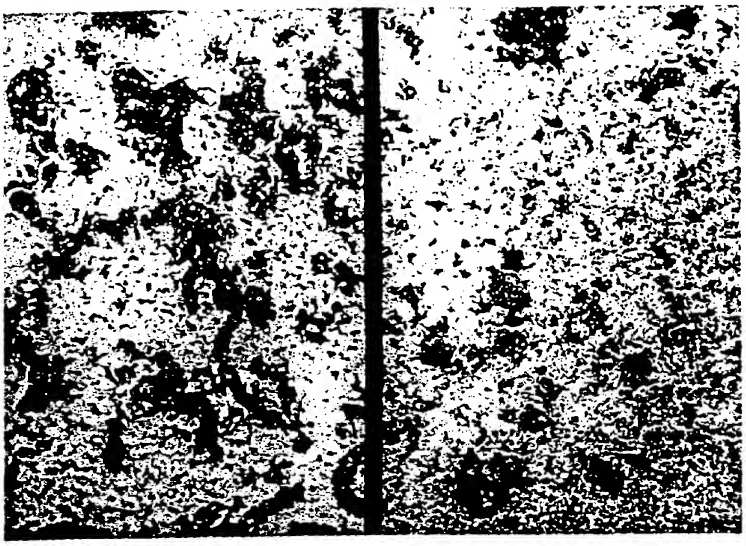

Figure 2 Phagokinetic track assay. MPs were allowed to migrate for 6 hr on matrices of native albumin (left panel) or AGE albumin (right panel) which had been coated with colloidal gold particles. The dark tracks made by locomoting MPs are visualized by darkfield microscopy. ×125

native albumin revealed minimal inflammatory infiltrate except at the tissue-tube interface (Fig. 3, left panel). In contrast, however, tissue from rats treated with AGE-impregnated PTFE tubes revealed a considerable inflammatory infiltrate, with mononuclear cells observed moving through the interstices of the tubes (Fig. 3, right panel). Many of the monocytes appear to have undergone activation, as evidenced by leukocytoclastic changes.

These data are consistent with the concept that AGEs may promote monocyte activation. In fact, previous studies have shown that AGE-monocyte interaction results in their activation (37–38), as manifested by production of cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) as well as the growth factor platelet-derived growth factor (PDGF).

Taken together, these data suggest a model in which soluble AGEs, such as those circulating in modified plasma proteins or those on the surface of diabetic red blood cells, may attract monocytes and mediate their migra-

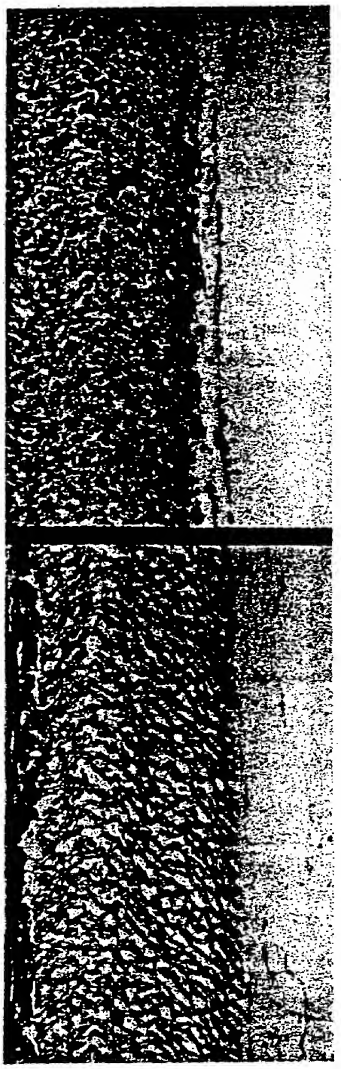

Figure 3 Implantation of PTFE tubes with absorbed AGE albumin into rats. PTFE tubes were impregnated with either native albumin (left panel) or AGE albumin (right panel), implanted subcutaneously into the backs of rats, and removed after 4 days and stained with hematoxylin and eosin. ×110.

tion. In contrast, immobilized AGEs, such as those formed/deposited in the vascular wall, may retard the migration of monocytes, a process which may ultimately lead to their activation, a process which may predispose to the development of vascular lesions.

IV. INTERACTION OF AGEs WITH ENDOTHELIAL CELL RAGE: CLEARANCE OF AGEs BY ENDOTHELIAL CELLS, INDUCTION OF CELLULAR OXIDANT STRESS, AND ENHANCED EXPRESSION OF VASCULAR CELL ADHESION MOLECULE-1

A. Endothelial Cell Handling of AGEs: Clearance Studies and Experiments Assessing Cellular Activation

Previous studies have identified the presence of RAGE in the vascular endothelium by immunohistochemistry using anti-RAGE IgG and by in situ hybridization (34). The endothelium is in a critical position to mediate the effects of circulating AGEs, either those that form on circulating plasma proteins or those that deposit/form on the surface of diabetic red blood cells with the rest of the cell. In addition to its potential role in processing/removing AGEs from the intravascular space, the endothelium may also function to mediate certain perturbations that are a consequence of their interaction with AGEs, all of which may alter the cellular phenotype, thereby predisposing to vascular dysfunction. In this context, our earliest studies on AGE-endothelial interaction demonstrated that when cultured endothelial cells (ECs) were exposed to AGEs, critical cellular properties were altered resulting in a procoagulant and more permeable phenotype (30). Specifically, we observed suppression of thrombomodulin, sustained induction of low levels of tissue factor, and increased diffusional transit of macromolecular solutes across the EC monolayer (30). The central role of RAGE in mediating the interaction of AGEs with the endothelium is suggested by studies which demonstrated blockade of binding of radiolabeled AGEs to cultured ECs (11) by anti-RAGE IgG(11).

It was therefore most important in this context to study the effects of AGEs infused into animals. Extensive studies with ^{125}I-AGE albumin administered intravenously into mice revealed that in contrast to radiolabeled native albumin, radiolabeled AGE albumin demonstrated an initial rapid phase of clearance, with greater than 50% of the protein being cleared from the vascular space within minutes after administration (39). Studies assessing tracer accumulation in the organs demonstrated that AGEs accumulated in the most vascular organs (39). Studies utilizing pretreatment of the animals with either anti-RAGE IgG or sRAGE resulted in a marked

delay in AGE clearance, suggesting that these reagents were capable of interfering with the interaction of circulating AGEs with EC RAGE (39).

Electronmicroscopic studies utilizing AGE albumin linked to colloidal gold revealed that in a murine coronary vasculature model, AGEs quickly interact with the EC surface, are subsequently endocytosed, and traverse the EC layer, in part by transcytosis, ultimately being released at the abluminal surface (39). As with the radiolabeled AGE clearance studies, this process was significantly inhibited in the presence of anti-RAGE IgG, whereas nonimmune IgG was without effect.

The importance of these results is stressed by studies in which infused, nonradioactive AGEs into mice resulted in increased mRNA for interleukin-6, again a process inhibited in the presence of RAGE blockade (39). Taken together, these studies suggest that AGEs are capable of interacting with EC RAGE, with potential consequences, including activation of cytokines, which may predispose to the development of vascular lesions. The central role of RAGE in mediating these effects is demonstrated by studies in which blockade of RAGE—either by blocking access to cell surface RAGE with anti-RAGE IgG or by binding to AGEs in the intravascular space thereby limiting their interaction with cell surface RAGE utilizing sRAGE—inhibits not only AGE clearance but, in this case, induction of, for example, mRNA for IL-6 transcripts.

B. Induction of Oxidant Stress in the Vasculature by Exposure to AGEs via a RAGE-Dependent Pathway

During the process of AGE formation, oxidation and generation of reactive oxygen intermediates are formed (9,10). We therefore postulated that, at least in part, one of the main mechanisms by which AGE-RAGE interaction might alter/perturb cellular properties is via generation of oxidant stress. Initial studies in tissue culture revealed that exposure of AGE albumin to cultured ECs resulted in the production of thiobarbituric acid–reactive substances (TBARS), induction of heme oxygenase mRNA, and activation of the transcription factor NFκB (17). In contrast, native albumin was without effect. All of these markers, indicative of enhanced oxidant stress, were inhibited in the presence of anti-RAGE IgG (17). Comparable results were obtained when diabetic red blood cells, not red blood cells isolated from normal individuals, were exposed to cultured ECs (35).

To better test the hypothesis that ligation of cell surface RAGE by AGEs delivered oxidant stress to the tissues, AGE albumin was infused into normal mice resulting in increased tissue TBARS, increased mRNA for heme oxygenase, and activation of NF-κB as assessed by electrophoretic mobility shift analysis of liver nuclear extracts (17,40). Again,

native albumin was without significant effect, and these processes in AGE-treated animals were inhibited by preinfusion of anti-RAGE IgG.

To detect these malondialdehyde-reactive epitopes in the vasculature itself, AGE albumin or native albumin was administered intravenously into rats, and after 1 h, samples were taken for immunohistochemical analysis using antibody that specifically recognizes malondialdehyde epitopes (17). Analysis of the lungs revealed vascular staining (in the ECs and vascular smooth muscle) of AGE-treated animals (Fig. 4, left panel) suggestive of enhanced oxidant stress. In contrast, no staining was observed in the pulmonary vasculature of animals treated with native albumin (Fig. 4, right panel). In the former case, nonimmune IgG was similarly without effect (17).

These studies were important to identify the site(s) where AGEs deliver oxidant stress after their infusion. Taken together, the data suggest that

Figure 4 Immunohistological detection of malondialdehyde-reactive epitopes in the vasculature of rats infused with AGE versus native rat serum albumin. Rats were infused with either AGE rat serum albumin (left panel) or native rat serum albumin (right panel). After 60 min, the animals were killed and lungs processed for the detection of malondialdehyde using indirect immunoalkaline phosphatase with antibody generously supplied by Dr. Joseph Witztum, University of California at San Diego. ×400.

AGEs interact with EC surface RAGE, and one of the consequences of this interaction is enhanced oxidant stress to the tissues, a process with multiple potential consequences. In this setting, one of the most important consequences may be activation of NFκB.

The implications of activation of the transcription factor NFκB are far reaching in this setting. Activation of this transcription factor has been linked to oxidant-sensitive mechanisms (40). Furthermore, its activation is probably important in the ultimate regulation of increased transcription of such entities as adhesion molecules, cytokines, or growth factors; all of which may be important in the pathogenesis of vascular lesions (41). In this context, we considered that enhanced expression of EC vascular cell adhesion molecule-1 (VCAM-1) might be a result of the interaction of AGEs with EC RAGE.

C. AGE-RAGE Interaction Increases EC Expression of VCAM-1

It is likely that many factors contribute to the increased and accelerated course of vascular disease in diabetic individuals compared with normal controls. One of the critical steps in this process, however, is likely to be the enhanced expression of adhesion molecules on the vascular endothelium, which results in the adherence of circulating blood cells to the vessel wall. In this context, previous studies have indicated that, at least in part, the transcriptional regulation of VCAM-1 is controlled by oxidant-sensitive pathways (42). We, therefore, considered whether AGEs interacting with their cellular receptor RAGE might result in increased expression and functional activity of VCAM-1.

In the setting of diabetes, VCAM-1 is of particular interest, as its expression has been identified in the early phase of experimental hypercholesterolemia-induced atherosclerosis (43–44). Furthermore, enhanced VCAM-1 expression has been shown in the vessel wall of rabbits rendered diabetic with alloxan (45), as well as in human atherosclerotic lesions (46).

Our data indicated that when either AGE albumin or AGEs immunoisolated from diabetic plasma were exposed to cultured human endothelial cells, ELISA of cellular extracts revealed increased cell-associated VCAM-1 compared with ECs treated with nonglycated proteins (47). In order to assess the functional implications of increased VCAM-1 on the cell surface, we treated ECs with either AGE or native albumin and subsequently performed binding assays using ^{51}chromium-labeled Molt-4 cells (cells which bear the counterligand for VCAM-1, VLA-4) and demonstrated significantly increased binding in the presence of AGEs (47). In both cases, the increased expression and function of cell-associated VCAM-1 was largely inhibited in the presence of RAGE blockade but not by control interven-

tions (47). In addition, both processes were inhibited in the presence of antioxidants such as N-acetyl cysteine. Consistent with these data, Northern analysis and nuclear run-on analysis demonstrated increased transcription of VCAM-1 in the presence of AGE albumin, not native albumin, a process which is inhibited by anti-Rage IgG.

Since previous studies have identified binding sites for NFκB in the promoter of VCAM-1, which have been shown to participate in the regulation of VCAM-1 in response to such factors as cytokines (48–49), at least in part, by oxidant-sensitive mechanisms. As demonstrated by electrophoretic mobility shift assay, in comparison with native albumin, nuclear extracts prepared from ECs exposed to AGE albumin resulted in an increased DNA binding activity for NFκB site in the VCAM-1 promoter (Fig. 5, lanes 2 and 3, 6, and 10, respectively). This binding was blocked in a dose-dependent manner in the presence of anti-RAGE IgG (Fig. 5, lanes 11 and 12) but not in the presence of nonimmune IgG (Fig. 5, lane 13). Similarly, pretreatment with N-acetylcysteine resulted in inhibition of AGE-induced activation of NFκB (Fig. 5, lane 14). Supershift studies using antibodies to p50 and p65 demonstrated that both members of the NFκB are involved in binding to specific sites in the VCAM-1 promoter (Fig. 5, lanes 7–9). These data suggest that at least of the mechanisms by which AGEs induce enhanced expression of VCAM-1 is via activation of this transcription factor.

Consistent with these in vitro studies, infusion of AGE albumin into normal mice resulted in increased immunoreactivity for VCAM-1 in the lung vasculature (Fig. 6, right panel). In contrast, infusion of native albumin was without effect (Fig. 6, left panel).

In addition to increased expression of cell-associated VCAM-1 in the presence of AGEs, one of the further interesting findings in this setting was the detection of VCAM-1 antigen in the supernatants of AGE-treated (as well as TNF-α–treated) ECs (47). We therefore inquired whether patients with diabetes had increased levels of soluble VCAM-1 (sVCAM-1) in their plasma compared with age-matched controls. In both cases, only patients with renal insufficiency were excluded from analysis. The level of sVCAM-1 in the plasma of diabetic patients was found to be 1,115 ng/ml compared with 632 ng/ml in the plasma of nondiabetic controls ($P < .05$). These data paralleled the changes in the levels of AGEs, as measured by AGE albumin equivalent in diabetic plasma compared with control plasma (244 ng/ml versus 157 ng/ml AGE albumin equivalents, $P < .05$) using an ELISA utilizing affinity-purified anti-AGE IgG (16,17,47).

Further analysis of sVCAM-1 in both AGE-treated EC supernatants and patient plasma demonstrated in immunoblotting studies that the immunoreactive material for VCAM-1 is intact, with an $M_r \approx 110$ kD. Fur-

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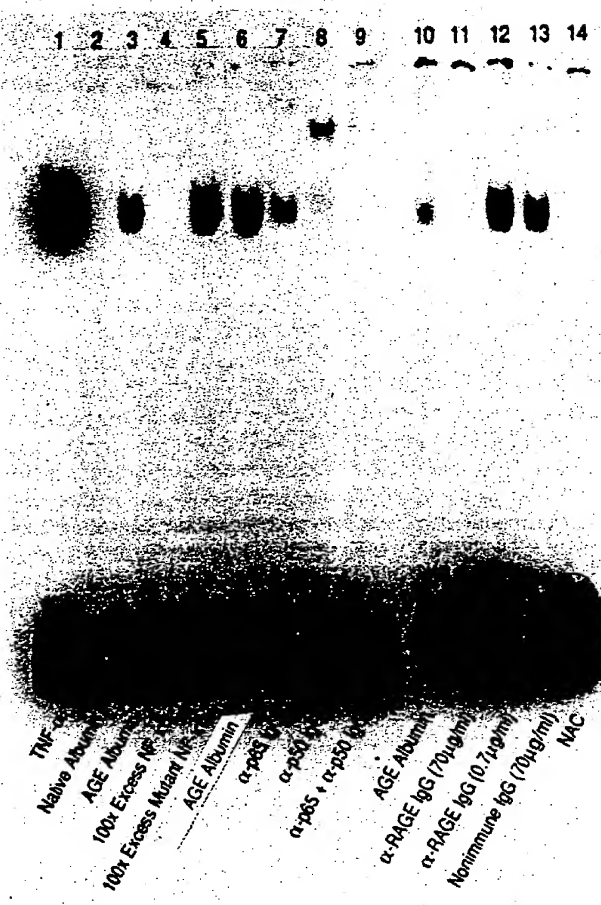

Figure 5 Binding of endothelial cell nuclear proteins to NF-κB binding sites in the promoter of VCAM-1: analysis by electrophoretic mobility shift assay. All lanes contain labeled probe as follows: lane 1, ECs were incubated with TNF-α for 6 hr; lane 2, nonglycated albumin; lanes 3, 6, and 10, AGE albumin; lanes 7–9, nuclear extracts of AGE-treated cells with anti-p50 IgG, anti-p65 IgG, or both anti-p50 IgG and anti-p65 IgG together, respectively. In lanes 11 and 12, ECs were pretreated with anti-RAGE IgG (70 vs 7 μg/ml, respectively) prior to the addition of AGE. In lane 13, ECs were pretreated with nonimmune IgG prior to AGE, and in lane 14, ECs were pretreated with N-acetylcysteine prior to AGE albumin.

Figure 6 Infusion of AGE albumin induces vascular expression of VCAM-1 in the pulmonary vasculature of mice. Mice were infused with either native albumin (left panel) or AGE mouse serum albumin (right panel). After 30 min, the animals were killed, lungs harvested, and immunostaining with antimurine VCAM-1 antigen performed. ×340.

thermore, no degradation was observed on the immunoblots (50), suggesting that VCAM-1 was released/cleaved from the cell surface in an intact form.

We hypothesized that elevation of sVCAM-1 might be, at least, a marker of endothelial perturbation in diabetic patients compared with control individuals and that its measurement might in fact be a marker of the effectiveness of therapeutic intervention. To further test this concept, we found that the levels of sVCAM-1 in diabetic patients with microalbuminuria were elevated compared with levels of sVCAM-1 in normoalbuminuric diabetic individuals (757 ng/ml vs 505 ng/ml, $P < 0.05$ [50]). The presence of microalbuminuria is of particular importance, since its presence in the diabetic patient has been linked to an increased incidence of cardiovascular morbidity and mortality, and it is considered a marker of diffuse vascular hyperpermeability (51–52). In fact, in our study, 5 of 12 patients with microalbuminuria had evidence of cardiovascular disease, whereas none of 8 patients with normoalbuminuria demonstrated abnor-

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malities of the cardiovascular system, $P < .05$ (50). Taken together, these data suggest that the enhanced presence of VCAM-1 in diabetic vasculature may be, at least in part, a reflection of the interaction of AGEs interacting with cell surface RAGE. One of the consequences of this interaction may be the increased adherence of mononuclear cells to the vascular wall, which is a process likely pivotal in the development of diabetic vascular disease.

V. BLOCKADE OF RAGE AS A POTENTIAL TARGET FOR INTERVENTION IN THE DEVELOPMENT OF VASCULAR COMPLICATIONS IN DIABETES

These data suggest that the interaction of AGEs with their cell surface receptor RAGE may be of central importance in the creation of an environment favorable to the development of vascular lesions. We have demonstrated that AGE-RAGE interaction is involved in the development of monocyte migration, as well as monocyte retention at sites of immobilized AGEs. Activation of monocytes is a likely sequela in this setting. RAGE has been demonstrated to be important in the clearance of AGEs from the intravascular space, and engagement of RAGE by AGEs results in the generation of enhanced oxidant stress in the vasculature. One of the consequences of this enhanced oxidant stress is likely to be the enhanced expression of cell-associated VCAM-1. These data further suggest that blockade of RAGE might ultimately be a target for therapeutic intervention in the setting of diabetic vascular disease. Indeed, recent findings have suggested that sVCAM-1 may have angiogenic properties, a situation to be of likely detriment in the diabetic vascular milieu, especially, for example, in such sites as the retina (53). Taken together, these findings suggest that blockade of AGE-RAGE interaction represents a logical target for therapeutic intervention in diabetic vascular disease.

In this context, our recent studies have demonstrated that infusion of sRAGE into diabetic rats reversed the increased tissue-blood isotope ratio (TBIR) observed in these animals, which is a marker of endothelial cell dysfunction (54–56).

In conclusion, these data suggest that further assessment of AGE-RAGE interaction and RAGE blockade in such models as wound healing and accelerated atherosclerosis will likely provide insight not only into the role of RAGE as a potential therapeutic target but will also aid in further dissecting the mechanisms set into motion on ligation of RAGE by AGEs or its other ligands.

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